ULTRAVIOLET SPECTROPHOTOMETRY CONSIDERED FROM AN INSTRUMENTAL STANDPOINT

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Chemisch Weekblad 49 (1953) 482 - 491

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II. CRITICAL INVESTIGATION OF SIMPLE SPECTROPHOTOMETERS

R. Schmidt

Chemisch Weekblad 49 (1953) 492 - 493

(From Dutch)

III. THE BEHAVIOUR OF THE SPEKKER ABSORPTIOMETER

R. Schmidt

Chemisch Weekblad 49 (1953) 494 - 496

(From Dutch)

IV. PRECISION EXTINCTION MEASUREMENTS WITH SIMPLE SPECTROPHOTOMETERS OF THE DEFLECTION TYPE

J. H. L. Zwiers

Chemisch Weekblad 49 (1953) 496 - 499

(From Dutch)

25X1

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J. van DRANEN

SUMMARY

This article deals with ultraviolet spectrophotometry from an instrumental standpoint, and discusses in particular the Beckman and Unicam photo–electric quartz spectrophotometers. The first section contains a short description of the construction and operation of these instruments. Thereafter, the accuracy of the extinction measurements which may be obtained with these instruments, is dealt with in detail.

INTRODUCTION

In this article, dealing with ultraviolet spectrophotometry, from an instrumental standpoint, we should like to discuss more particularly the Beckman and Unicam photo–electric quartz spectrophotometers.

Firstly, however, the advantages and disadvantages of the different methods of measurement will be briefly discussed. The radiometric methods, by which the intensity of the radiation is determined by bolometers or thermopiles, are not of importance for ultra-violet photometry, because the useful wavelength range falls within the infra-red region. Photographic and photo–electric methods, however, are all used for the measurement of visible and ultraviolet radiation. The properties of these methods are mentioned in Table 1 (1)[*]. Although, from this table, it appears that no one of the methods is superior, the experience of the last decade has shown that modern photo–electric instruments possess very considerable advantages over photographic for the measurement of extinction curves in the ultraviolet region. This improvement in technique is stressed by examining the difficulties discussed in the dissertation by Dr. C. W. F. Spiers (Amsterdam 1936) in obtaining accurate measurement of the absorption spectra of pyridine and its homologues, compared with the ease with which this can be achieved, at present, with for instance a Beckman instrument.

MEASUREMENT OF INTENSITIES

METHOD	WAVELENGTH RANGE	PANATOMIC PROPERTY	CUMULATIVE PROPERTY	LINEARITY
Visual	4000 – 7000 Å	limited	none	very poor
Photoelectric	to 30,000 Å	none	fair	good
Photographic	to 12,000 Å	excellent	good	poor

The disadvantages of the photographic method compared with the photo–electric method are:

1. Difficulty in adjusting the spectrographic apparatus.

2. Establishing the extinction curve from photographic records:

    (a) Optically – inaccurate
    (b) Photometric – slow.

3. Expensive, especially if the extinction is to be measured at single wavelengths only.

4. Operators have to be highly trained.

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[*] For references, see page 13.

I. THE MODERN QUARTZ SPECTROPHOTOMETER

H.H. Cary and Arnold O. Beckman, the designers of the "Beckman", in an article in the Journal of the Optical Society of America (2) have thoroughly explained the motives and experiments which led to the construction of their instrument. Their aim was to develop an instrument, which would satisfy the conditions of economy, accuracy and simplicity of handling. A compromise, as favourable as possible, was sought between these somewhat contradictory requirements. The photoelectric system was chosen; the following fundamental conditions must be satisfied if accuracy is required:-

1. The illumination must be monochromatic as far as is possible.

2. The intensity of the monochromatic beam must be sufficient for accurate measurement.

These requirements determine the selection of the four principal components of the instrument:-

(a) Source of light;

(b) Optical system;

(c) The photo cells; and

(d) The electrical system. (See Figures 1 and 2).

(a) LIGHT SOURCE

A 25 watt, 6 volt tungsten lamp is used as a light source for the region between 10,000 $\overset{\circ}{A}$ and 3,200 $\overset{\circ}{A}$, the current being supplied by an accumulator. Below 3,200 $\overset{\circ}{A}$, the continuous spectrum of a hydrogen discharge tube is used. In this case an electronic voltage stabiliser is used (stabilisation $\pm$ 0.2%) to supply the required 0.4 amp. From A the light falls on the focussing mirror B (aluminized, focal length = 2.85 cm) and is reflected from mirror C on to the slit D. The slits (curved, bilateral, 13 mm high) can be accurately adjusted between 2.00 and 0.01 mm: the entry slit is below the exit slit. Calculation and experiment have demonstrated that the light on the entry slit has its origin in a small section 0.6 mm wide and 3.1 mm high of the source of light, thereby satisfying the requirements for high intensity and small area. From this it follows that with the aid of B and C a seemingly small source of light is in a position to supply all the light required by the monochromator.

(b) OPTICAL SYSTEM

In the construction of their monochromator (that part of the instrument D, E, F., which receives light of a very definite wavelength from the original light source A) Cary and Beckman were confronted with the difficult choice of using a grating or a quartz prism. The advantage of a grating is in general, the greater dispersion, which means that the slits and the wavelength scale need not be made so accurately. Furthermore the price of a replica grating is lower than that of a quartz prism. However, the principal reason for deciding to use a prism, lay in the fact that a grating scatters much more light.

It is most important that the monochromatic light should be free from stray light: although, in a well constructed monochromator, the stray light, which originates from undesired reflections, can be made negligibly small, the surface of a grating is so much less optically perfect than a prism that stray light, not originating from reflection is much increased. Extensive tests undertaken by these investigators with eight different gratings and two different prisms in different installations, showed on the whole that scatter with a grating is ten times greater than with a prism. For the optical system, the so-called Littrow installation was finally chosen with mirror collimation, which requires a minimum in optical and mechanical details: The collimator E (aluminized, focal length = 50 cm) in whose focus the slit D is situated, reflects rays deviating 5° from the principal axis, whereby both astigmatism and spherical aberration occur. The spectral width of the band of light emerging at D is thereby increased by an amount which would be obtained by increasing the width of the slit by 0.02 mm. We shall return later to a simple formula for calculating the spectral band width for a given slit width. From E the light falls on the prism F. This reflecting prism (refracting angle 30°, the aluminium layer on the back has a diameter of 5 cm) is made from very good quality crystal quartz. Compared with a monochromator with a 60° prism, only half the quantity of quartz is necessary. Furthermore, the prism need not consist of two halves of opposite

rotatory power in order to avoid doubling of the image, due to the double refraction
of the quartz. The prism can be rotated by means of a long spindle, the other end of
which is connected to the wavelength scale. This scale consists of a quadruple spiral
one metre in length reading from 2,000 Å to 2 μ. The light, which comes via F and E from
the exit slit at D is not blocked by the mirror C which is situated below it, but passes
directly through the cell G containing the absorbent substance. The intensity of the
unabsorbed light is then measured by the photocell H. The wavelength of the light which
traverses the path D, G, H is indicated on the wavelength scale. By rotating the prism
F, it is possible to adjust to any desired wavelength.

The use of a mirror as collimator obviates the need for an expensive achromatic lens,
while focussing also becomes much simpler.

(c)   PHOTOCELLS

Two different photocells are used in the instrument: a red sensitive cell and an
ultraviolet sensitive cell. The first is a caesium oxide cell, which must be used above
6,250 Å. Though this cell is useful down to 4,000 Å, below 6,000 Å, stray light, to which
this cell is exceptionally sensitive, begins to play a greater role, so that it is better
to use the ultraviolet sensitive caesium antimony cell.

(d)   ELECTRIC SYSTEM

In an article of restricted length, it is of course not possible to go fully into
the question of the rather complicated circuits. We reproduce, therefore, a simplified
circuit diagram given by Caster (4) with which to explain the measurement of the photo-
current (Figure 2).

The photocurrent is measured by compensating the voltage drop across the resistance
R of 2×10^9 ohm with a potentiometer. This operation is controlled by a galvanometer
through an amplifier. The amplifier has an amplification factor of 5×10^7, by means of
which full deflections of the galvanometer are possible with a light influx of 2.5×10^{-8} watt,
corresponding to a photocurrent of 10^{-13} amp. The resistance of the photocell varies according
to the intensity of the focussed light from 10^9 to approximately 10^{13} ohm. The photocell
compartment contains a dessicant to prevent leakage with these high resistances. By means of
P it is possible to switch from one photocell to the other.

A point between the photocell and the galvanometer is maintained at + 20 volt. The
potential thus drops from + 20 to + 2 volt across the galvanometer and valve T. The current
passing through the galvanometer thus depends on the grid voltage of this valve. In the
other branch of the circuit, the same drop occurs across the photocell and resistance R.
Now if light falls on the photocell, the internal resistance of the cell is lowered, and thus
the grid voltage increases. In order to compensate for this change, the total potential
difference of the system can be adjusted by means of the potentiometer knob which has a scale
readable in percentage transmission (and optical density or extinction D, $D = - \log T$). The
variation in the photocurrent is thus directly balanced by this potentiometer. The dark
current is the small residual current, which also occurs when the photocell receives no light.
In order to compensate for this, the cell and the potentiometer are disconnected; after that
the auxiliary potentiometer is adjusted by means of the dark current control knob, so that
the galvanometer is at zero.

The third potentiometer, the "sensivity potentiometer" is used for compensating the
absorption of the solvent. By taking this as a zero level, this part of the absorption for
which the dissolved substance is responsible can be measured directly. The transmission
potentiometer can be disconnected by setting the switch at the "check" position. The solvent
is in the path of the light. By means of the sensitivity knob the galvanometer is set at
zero (to save time, the transmission potentiometer is replaced by an equal resistance, so
that the transmission need not first be set separately at 100%). Should the transmission
be less than 11%, it is better to set the switch at 0.1. The scale values will become ten
times as small, thereby increasing the accuracy of reading.

For biochemical purposes, it is important to be able to work with very small quantities
of liquid. Lowry and Bessey (5) could indicate in 0.01-0.05 cm^3 serum, the vitamins A and C
in fractions of γ's.

- 4 -

OTHER APPLICATIONS

The Beckman spectrophotometer can also be made suitable with relatively few alterations, for a number of other purposes, besides the measuring of the extinctions of solutions. These will be briefly described.

Greater quantities of liquid and gases (optical length of path of the gas cell 10 cm) can be measured in containers obtainable separately. The only alteration is, of course, that another cell must be used for these containers.

In addition special apparatus can be supplied for fluorescent and flame photometry. The difficulty which presents itself with this fluorescent apparatus is that while avoiding the monochromator the light falls directly on the photocells. Thus the total intensity of the fluorescent light is calculated, and because of this it is not possible to analyse an unknown combination or to define a mixture of fluorescent substances. Dr. A.M.W. Indemans (28) drew our attention to a method, by which the monochromator can be used for the determination of the complete fluorescent spectrum. The fluorescent light, generated by a mercury lamp, passes via a converging lens directly to the mirror C, Figure 1. Therefore it is not neceesary to introduce still further alterations (6). In the case of very weak fluorescence, the photocells can be substituted by photomultipliers.

In order to make the instrument suitable for flame photometry, the lampholder must be substituted by a gas-oxygen flame. In this case the liquids are disposed in the customary way.

THE UNICAM PHOTOELECTRIC SPECTROPHOTOMETER

This instrument presents only a few minor items, which differ from the Beckman. It may be said, in general, that the Unicam is designed to be somewhat more robust than the Beckman. The control panel slants back for better visibility unlike the Beckman, where one must look up at it. At present, in order to avoid this last disadvantage, we have fixed the light in the Beckman in a position slanting towards the observer. In the Unicam, the lampholder contains the tungsten as well as the hydrogen lamp. The condensor mirror B, Figure 1, can be focussed on the required lamp by means of a small lever.

The Hilger Uvispek (ultraviolet and visible photoelectric spectrophotometer) and the Zeiss Opton Spectral Photometer (with monochromator and an electron multiplier in place of photocells) belong to the same group as the Beckman and the Unicam with regard to cost and capability.

In contrast to the Beckman and the Unicam, separate accumulators for these two last mentioned instruments are not required, supply of an alternating current source being sufficient. Furthermore, the differences in light source, optical system and suchlike, are not great. The Uvispek also possesses the same compact construction as the Beckman. In the Zeiss, the separate components are arranged on an optical bench. The wavelength scale, however, is read in an entirely different way to that of the other instruments. In this case an image of the entrance slit is shown on the scale. The centre of the image indicates the wavelength, the width shows the spectral bandwidth.

In order to give an opinion, as to which of the four instruments is the best, a very extensive investigation would be necessary. For this investigation, one ought to have at one's disposal at least 10 specimens of each instrument. A priori, one may expect that these instruments, obtainable practically at the same price and supplied by very good firms, would not show any great differences in quality. Each apparatus can be delivered with quartz optics (range approximately 2,100 Å - approximately 10,000 Å, see also discussion) as well as with glass optics (range approximately 3,800 Å - approximately 11,000 Å) and provided with the necessary accessories for fluorescence, reflection measurements and flame photometry.

"AUTOMATIC" SPECTROPHOTOMETERS

In the instruments previously discussed the extinction curve must be recorded point by point. By making use of modern electronic resources it is now possible to record the curve automatically. In the Cary Recording Spectrophotometer (7), the light of the hydrogen or tungsten lamp is split into two beams, of which one is the "100% transmission" beam the remaining beam falling on the material under measurement. The intensity of both beams is measured by separate photomultipliers. After amplification, the ratio of the two photoelectric currents is registered directly on an "electronic recorder". The double monochromator with two 30° quartz Littrow prisms gives the instrument a high resolving capacity, so that for

instance the rotation structure can be seen very well in the ultraviolet absorption spectrum. The spectral bandwidth in the ultraviolet region, except at the boundaries, is approximately $1/\text{Å}$. Kaye and others (8) have also succeeded in making the ordinary Beckman apparatus automatic, at the same time increasing the wavelength range considerably (to 2,700 m μ). In this way the red sensitive cell is replaced by a lead sulphide cell and the cell, sensitive to ultraviolet by a multiplier; the beam of light is modulated in order to increase the amplification. The resolution of the instrument thus modified is ten times higher than that of the original instrument, so that one is really able to get the mercury line split up at 2,541 Å, although the distance between the components is only 1.7 Å. The saving of time is considerable; in ten minutes, the whole infra-red spectrum of 700 m μ to 2,700 m μ can be recorded. These three advantages: greater wavelength range, saving of time and greater resolution in the ultraviolet region, make this automatic operation very attractive, especially as an existing instrument can be modified. Important details on the subject of the instrumentation of spectrophotometers can be found in recent numbers of the Review of Scientific Instruments and the Journal of the Optical Society of America. We also refer to the well known work compiled by Mellon: Analytical Absorption Spectroscopy (9).

THE STRUCTURE OF THE EXTINCTION CURVE

Here we would like to mention briefly a few of the all important facts from theory. Naturally the most important application of the ultraviolet absorption spectra is the identification and qualitative (quantitative) analysis of chemical compounds. The more simple infra-red or Raman spectra lend themselves better to the examination of the structures of molecules.

The energy of a molecule, may be regarded as consisting of electron energy, vibration energy and rotation energy. A change in the electron energy takes place due to absorption of light by the molecule. As a result, the molecule moves from the basic position to a new one. In a physical sense this means that a particular electron makes a jump towards a band with higher energy (this is entirely analogous to the absorption of light, as described with the aid of the atom model of Bohr). Electronic bands are produced when there is a change from one electronic level to another. The separate bands are caused by a jump in the vibration energy which is superimposed on an electron jump. The vibration energy is the energy of vibrations which the atoms execute with respect to one another. With some reserve, it can be said that these vibration bands are often the peaks in the extinction curve, obtained with a spectrophotometer. The delicate structure of these bands is in turn caused by jumps in the rotation energy. Experimentally, this rotation structure can be indicated only for the absorption spectrum of a gas. In the fluid and solid states, the rotation is hindered or even completely prevented by the molecular interaction force, in such a way, that the rotation energy conditions are spread out to a continuum. We can therefore state that the energy of the electron jump determines the position of the centre of gravity of the band system. The width of this depends on the vibration energies.

Before formulating this somewhat more quantitatively, it must, at first, be mentioned that in spectroscopy, it is customary to express the energy of a vibration by the wave number. The wave number is the frequency ν divided by the velocity of light C. If the wave number of a vibration is ν, then the corresponding energy, according to Planck's ratio is: $h \cdot \nu = h \cdot \nu \cdot C$ per grammolecule $N \cdot h \cdot c \cdot \bar{\nu}$ or calculated 2.844 $\bar{\nu}$ cal/mol. The energy E of an absorption at 5,000 Å is thus: $\lambda = 5,000$ Å $= 5 \times 10^{-5}$ cm; $\nu = C/\lambda$ or $\nu = 1/\lambda = 20,000$ cm^{-1} or $E = 2.844 \times 20,000 = 57$ Kcal/mol. (1 Kcal/mol = 362 cm^{-1}).

The construction of a band system is given below. The indicated boundaries are very rough and are more applicable to the organic molecules, which absorb ultraviolet in this region. As an aid to visualisation it can be said that the distances between the bands consist mostly of a few tens to hundreds of Å's; the rotation fine structure contains components of one Å up to at the most a few Å's.

BAND SYSTEM[*]

Position of band system determined by electron jump $\approx$ 3000 cm^{-1} (3333 Å)

The bands (spreading) due to vibrations $\approx$ 300-3000 cm^{-1}

Fine structure rotation bands

in gaseous state: instrument with wide dispersion, separate lines at small distances (order of magnitude 1 Å) $\approx$ 3-30 cm^{-1}

in liquid state: due to disturbance the separate points are linked together into a continuous line (extinction curve)

[*] The numbers indicate order of magnitude.

- 6 -

Let us discuss, for example, the well known absorption band system of benzene at 2,600 Å. Benzene consisting of 12 atoms, possesses 12 x 3 = 36 degrees of freedom.   In order to discover the number of vibrations of this molecule, one must subtract from this quantity the degrees of freedom of the translation (3) and of the rotation (3, for a linear molecule 2).   Therefore benzene possesses 30 vibrations.   Due to the great symmetry of the molecule, there are only 20 different frequencies, and of these, the so-called double damped vibrations coincide in pairs.   However, in this example, we are dealing with only two of the 20 frequencies.   In theory, the electron jump which occurs as a result of adsorption, is a forbidden transition, which is in accord with the six-sided symmetry of benzene.   The pure electron jump at 2620 Å therefore rarely appears in the extinction curve, but the band E (2590 Å) will, because besides the absorption of the electron system, the E vibration also absorbs a quantum.   This vibration (E indicates that the vibration is double damped) therefore passes from its basic state to its first adopted state.   Expressed in cm^{-1} the difference, between the rarely occurring O band and the E band of frequency of the E vibration amounts to 606 cm^{-1}.   The other bands now come into existence, because the A vibration absorbs one ($E + A$), two ($E + 2A$) or three ($E + 3A$) quanta.   Naturally the E vibration must always be associated with one quantum.   It is interesting that in the adopted state, the frequencies of A and E are smaller.   This is due to the fact that in the adopted state, the cohesion between the atoms has become weaker, because the loss of the electron has decreased the cohesion in the new state.

In the majority of cases, the absorbed light energy is transformed into heat.   At first the excited molecule transforms its surplus electron energy into vibration energy, because the electron system is energetically linked with the vibration.   By means of collisions between the molecules of the solvent, this vibration energy, in the case under examination, becomes distributed throughout the total fluid.   Therefore, it appears that the chance of fluorescent radiation is determined by the intensity of association between the electron system and the vibrations and the number of collisions.   Therefore, it is necessary for the occurrence of fluorescence, that the absorption electron system is reasonably protected against interior and exterior disturbance, so that the actuated molecule may remain for at least 10^{-8} sec. in an adopted state.   In addition to the wavelength of the absorption, the intensity, i.e. the value of the extinction coefficient, is of great importance.   Theoretically one can deduce intricate formulas for this coefficient. The theoretical values, however, may vary by a factor of 2 or 3, from the experimental results which even under unfavourable circumstances, are accurate to within 20%.   For a book containing many useful particulars on the subject of optics, emission and absorption of light, we would like to refer to E.J. Bowen "The chemical aspects of light" (10).   This work, written on an academic level, does not demand any special pre-knowledge of the subject.   Friedel and Orchin have published a collection of nearly 600 ultraviolet spectra of aromatic compounds (11).   This collection if added to in future, as is the intention, will be of very great importance to users of spectro-photometers.   The introduction contains a useful review of instruments and the use of ultraviolet spectra for analytical purposes.

II.   THE ACCURACY OF THE EXTINCTION MEASUREMENTS

Having discussed the instruments and a few theoretical aspects of the ultraviolet spectra, we will now deal with the question of accuracy.   Publications on this subject have not been lacking during the last years, so now that the first enthusiasm for these spectrophotometric instruments is a thing of the past, we have made a more critical examination.   It is a fact that some investigators have a very high opinion of the accuracy reached.   The literature contains values such as 14704 and 4954 for the extinction coefficient.   It is obvious this magnitude cannot be determined so exactly, but, before going any further into this, we would like first of all to mention the most important results of very fundamental research, which Gibson and Balcom have undertaken, for the National Bureau of Standards, regarding the properties of the Beckman spectrophotometer (12).   This article is very highly recommended to anyone interested in spectrophotometry.

MULTIPLE REFLECTIONS

These are due to light which is reflected back and forth between the cell and the quartz slit.   If one measures a solution against the solvent (transmission set at 100%) then this error is generally negligibly small.   However, if the transmission of a glassplate is determined from the unweakened beam, the transmission may be up to 1% too high.

STRAY LIGHT

Gibson and Balcom mention that one can observe the stray light directly by visual means, if with the monochromator set above 7,000 Å or below 4,500 Å, one looks directly into the exit slit. According to these investigators, practically all energy above 1,200 mμ, as measured by the photocells, can be accounted for by stray light. Therefore the instrument cannot be used for wavelengths between 1,200 mμ and 2,000 mμ. They consider it necessary to use a Corning No. 9863 red purple filter, when using the tungsten lamp below 4,000 Å. This filter is placed between the slits and the cell. For measurements between 4,000 Å and 5,000 Å, it may be sometimes useful to fit a blue filter in the (third) open space of the slide.

FLUORESCENCE

If the dissolved matter shows any fluorescence, then naturally a small part of this fluorescent light also falls on the photocell. In view of the fact that the intensity of the fluorescent light is low this error may nearly always be neglected. In special cases suitable filters can be used. From the small quartz plate, which covers the entrance slit, the optics run parallel with the side-panels, and this may produce a polarisation effect. In order to prevent errors in the measurement of polarising materials, one should therefore remove the small plate, or else carry out the measurement with a different orientation.

THE WAVELENGTH SCALE

With the aid of the screw, which is situated on the left side panel and is connected to the collimator, the wavelength scale is calibrated to the green mercury line (5461 Å) with subsequent checking by the location of the spectral lines of hydrogen, helium and neon sources on the scale. Up to 6,000 Å, the error appeared to be not greater than 5 Å: while throughout the useful range the error nowhere greatly exceeded 10 Å. Therefore if calibration can be carried out by means of the green mercury line, this error can be ignored in most types of measurement. Only for very accurate measurements must the scale be calibrated at other points as well. It then becomes necessary to fix to the wavelength scale a small plate with an indicator strip, in order to prevent parallax. Such a device may be useful for everyday measurements also.

SPECTRAL BANDWIDTH

It may be said that the slit is the most important component of an optical instrument, as the slit is finally the indirect source of light, from which the spectrum is obtained. Every irregularity in the slit expresses itself in the image obtained, so that very high demands are required from the finish of the edges. It is very important that monochromatic light is used for the correct determination of the extinction curve of a solution, since Beer's Law only applies to monochromatic light. The most important magnitude for the monochromator used in spectrophotometers, is the spectral bandwidth, i.e. the narrow band of frequencies, which for a predetermined setting of the prism leaves the exit slit and falls on the cell. If the extinction coefficient ϵ is measured at a strict maximum or minimum, then, of course, the value of ϵ is very dependent on the spectral bandwidth. We give, by way of example, a number of observations of Hogness and others (13) for benzene in iso-octane for the wavelengths 2,540 Å (strict maximum) and 2,525 Å (strict minimum). It will be noted from this small list, that, as the spectral bandwidth becomes greater, the values $\epsilon_{max.}$ and $\epsilon_{min.}$ approach one another, because then in both positions, the monochromator transmits the same spectral region.

EXTINCTION COEFFICIENT OF BENZENE IN ISO-OCTANE

SPECTRAL BANDWIDTH	2,540 Å (max)	2,525 Å (min)
5.6 Å	212	45.3
8.8	204	45.3
16.0	190	45.5
32	154	50.8
80	104	62.5
120	89	69.0
160	77	72.2

- 8 -

Following up the work of Hardy and Young (14), Eberhardt (15) has derived correction formulas, making use of this dependence of ϵ on the spectral bandwidth, in order to define the true value of ϵ. However, a number of assumptions must be made, concerning the behaviour of the extinction curve in the neighbourhood of the maximum (for instance acute or parabolic angle). Much work can be saved by the application of this formula. In the case of the absorption spectrum of $K_2Cr_2O_7$ with flat maxima, the influence of the spectral bandwidth is much smaller than, to give an extreme example, rarely encountered absorption spectra with pronounced peaks. It follows that it is very important to state the spectral bandwidth when giving absorption spectra data in order to facilitate reproduction of the results. For the Beckman and the Unicam a simple formula is available for the calculation of the spectral bandwidth W, for a given slit aperture X (in mm's), namely, $W_S = 2 W_E = 2 W_D (X + 0.02)$. W_D is the nominal bandwidth per 1 mm. slit aperture. This factor is given by the designers in the form of a curve as a function of the wavelength (the nominal bandwidth is the wavelength range, for which the intensity of the light has dropped to one half of the maximum value).

λ	X (mm)	W_D (Å)	W_S (Å)
H_g-lamp 2200 Å	2.00	14.5	58^5
2500	0.60	23	28^5
3000	0.40	41	34
3500	0.30	68	42
T-lamp 3500	0.10	68	18
4500	0.04	15	18
6000	0.04	34	40
8000	0.05	66	92
1000 mμ	0.50	88	90 mμ

As has already been explained, the term $0.02 W_D$ is due to optical aberrations. The fact that the same formula can be applied to both the Beckman and the Unicam, is further proof of the similarity of these instruments. Information is given concerning the spectral bandwidths for a number of usable slit widths. For small wavelengths, it is possible to work with narrower slits, but the quantity of light which falls on the photocells then becomes too small and the transmission cannot be measured with sufficient accuracy.

THE MEASUREMENT OF EXTINCTION (OPTICAL DENSITY)

In order to know to what degree of accuracy the extinction can be measured it is necessary to examine the method of measurement. In order to obtain the extinction of a solution at a particular wavelength, the galvanometer must be set 3 times at zero.

(1) the "dark current" must be compensated.

(2) the transmission of the solvent is set at 100% by adjusting the slit and the sensitivity knob, or a fixed slit is used and regulation obtained entirely by means of the sensitivity knob, or the sensitivity knob is turned three times to the right and the indicator set at zero by adjusting the slit. The sensitivity control can then be re-set with precision.

(3) the switch is then set to 1.0 and the solvent is brought into focus by means of the transmission control. The indicator is then reset at zero. A small error is involved in each of these three steps. In addition there are fluctuations of the light source, which amount to 0.1% to 2%, according to Edisbury (16), dark current and sensitivity errors amount to 0.1%. A more detailed observation of the fluctuations in the reading of the optical density potentiometer is given by Edisbury.

The optical density potentiometer contains approximately one thousand turns giving an error of approximately 0.1%, as the contact arm must jump from turn to turn and therefore there is always a difference of a whole turn. For transmissions in the useful region, the following errors are calculated: $\Delta T = 0.001$ is constant. The error is defined as half the difference between two successive values.

TRANSMISSION T	EXTINCTION D $(D = -\log T)$	ERROR IN D $(\pm \Delta \frac{D}{D} \times 100\%)$
0.630	0.2007	
0.631	0.2000	0.18%
0.370	0.4318	
0.371	0.4306	0.14%
0.199	0.7011	
0.200	0.6990	0.15%

As is known, a small variation in the extinction coefficient gives the greatest variation in T at 37% transmission. The minimum uncertainty in D is therefore approximately 0.14%. From the fact that the needle of the galvanometer moves continuously, instead of swinging violently backwards and forwards as the control is adjusted it follows that this uncertainty is smaller than the limit of error in reading the galvanometer. According to Edisbury, it may be concluded, from analogous considerations, that there is an error of the same order of magnitude with regard to the "dark current" and the "sensitivity". Although this reasoning does not appear to us entirely conclusive, it is, however, not easy to find arguments against it. Naturally much depends on the accuracy, with which the galvanometer is set up. The pointer should always be adjusted from the same direction with regard to the zero point and it is more important to obtain the same reading for all three calibrations than to obtain an absolute setting of zero. Edisbury's observations lead to a total error of approximately one half percent in the "optical density" and the extinction coefficient, an error, which cannot be avoided with the present design of instrument. It is interesting to see what results are obtained in practice. Ewing and Parsons (17) have compared observations on 10 Beckman instruments. The extinction coefficient of the acid K phtalate, which can easily be obtained in a pure state, was measured at 281 m μ (maximum) and 264 m μ (minimum). For log ϵ (max.) the values varied from 6.45 to 6.17 (average value, 36 measurement with 10 instruments 6.314);; for log ϵ (min.) from 4.27 to 4.06 (average value 4.175). The values for single instruments amounted to: 6.39, 6.38, 6.39 and 6.40 for the "best" instruments; 6.26 and 6.31 for "average" instruments; 6.45, 6.41, 6.37 and 6.37 for the "worst" instruments. These observations show that the agreement for readings from a single instrument is much better than between different instruments (namely ± 1% against ± 4%). This is due to the fact that it is not entirely possible to avoid small variations in the manufacture of such a sensitive instrument as a spectrophotometer. Ewing and Parsons suggest therefore that each apparatus should be compared with a statistical average obtained from a large number of instruments and a correction table provided for each instrument. Now if consideration is given to the diversity of the sources of error, it is more than probable that the correction terms will be based on a degree of accuracy, which does not in fact exist. The British "Photoelectric Spectrometry Group" carried out in 1950 a comparative test, in which the extinction of solutions of $K_2Cr_2O_7$ in 0.01 N H_2SO_4 was measured with 63 instruments (35 Beckman, 15 Unicams and 13 Hilger Uvispeks).

The variation coefficient, that is, the value of the standard deviation expressed in percentages of the accepted value, for the measurements of 8 extinctions (2 different solutions with 4 wavelengths) amounted to 1.85%. It was once thought that photoelectric instruments tend to register low extinctions somewhat too high and the high extinctions somewhat too low. This test shows that this is not the case. Insofar as can be judged from the results of the 3 photographic instruments examined, it appears that these instruments did in fact suffer from this fault since they measured the extinctions a few percent too low. From statistics, it appears that a photoelectric reading is three times as accurate as a photographic reading. The following table indicates by how much actual measurements may vary from the accepted value. The extinction is expressed as E (1% 1 cm) that is the extinction obtained from a 1% solution with an optical path of 1 cm, a method often followed for natural products and polymer compounds, for which the molecular weight is not known. The accepted value for E (1% 1 cm) is compared with the average of the 2 highest and of the 2 lowest results for the 4 different wavelengths.

N. T. Gridgeman, has published the results for these 63 instruments statistically and suggested the use of $K_2Cr_2O_7$ as a standard (3 times crystallised from double distilled water and dried at 400°C; to this solution is added 0.25 ml. concentrated H_2SO_4 per liter). The extinction coefficient for $K_2Cr_2O_7$ is not too high or too low. There are four useful wavelengths (compare the small list) which cover the whole ultraviolet. For these wavelengths the extinction curve possesses apparently flat minima and maxima, so that the instrument can be focussed satisfactorily.

WAVELENGTH	ACCEPTED VALUE	E (1%, 1 cm) AVERAGE OF TWO HIGHEST	$K_2Cr_2O_7$ AVERAGE OF TWO LOWEST
2350 $\mathring{A}$ (min)	124.8	131.6	114.9
2570 (max)	145.2	150.0	138.0
3130 (min)	48.7	50.2	44.7
3500 (max)	106.7	108.3	105.2

K. F. Westerling and F. Hartog (Laboratory for General and Inorganic Chemistry and Laboratory for Analytical Chemistry, University of Amsterdam) have measured the extinction of solutions of $K_2Cr_2O_7$, $CoSO_4$ and $CuSO_4$ with the aid of a Beckman and a Unicam. The provisional results of this investigation are in agreement with the values given by other workers. For seven different solutions of $K_2Cr_2O_7$, $CoSO_4$ and $CuSO_4$ with extinctions between approximately 0.1 and approximately 0.9 (21 solutions in all) the deviation in the values measured by both instruments, was on an average a good 2%.

PRECISION COLORIMETRY

The photometric analysis methods may be more sensitive and quicker than the classic gravimetric methods, the disadvantage being that they are more inaccurate. Bastian et al (18) Ayres (21) and Hiskey et al (22) have developed differential or precision method of colorimetry, which reduces this disadvantage considerably. In principle precision colorimetry is based on the fact that the extinction of an unknown solution is compared with that of a known solution, for which the extinction coefficient differs but little in value. It will be shown that in this case the accuracy of the calculation will be much greater. The extinction E (optical density) of a solution = $\epsilon\,C\,l$; an identical increase ΔC in C always gives the same augmentation in the value for the extinction regardless of the value of the concentration C if the validity of Beer's law is assumed. By using high concentrations, the error $\Delta C/C$ could be made smaller if it were not for the fact that these high values for the extinctions can only be determined with much less accuracy than lower values (24). According to the above mentioned authors, it is better not to set the transmission for the pure solvent at 100%, but, for a solution with a concentration C_0 to use a neighbouring value C_x. If the difference $C_x - C_0$ can be measured accurately at for example 0.5% then the accuracy obtained is equal to $C_x - C_0/C_x$ x 0.5%. The following table gives a few results obtained.

COMPARISON SOLUTION (TRANSMISSION 100%)	UNKNOWN SOLUTION	DETERMINED WITH PRECISION COLORIMETRY
1.5000 g Cu/100 ml	1.6471 g	1.6467 g
0.10000 $N\ K_2Cr_2O_7$	0.09893 N	0.09897 N
0.10000 $N\ KMnO_4$	0.09891 N	0.09878 N

However, the question is whether this precision method can be used universally. Naturally it is always possible to find concentrations in the neighbourhood of the unknown and interpolate. The best way is to use a number of concentrations, both greater and smaller than the unknown. This gives complete freedom from deviations of Beer's law and similar laws. The weakest solutions obtained of a small concentration range are set at transmission 100% $(E = O)$. With high concentrations large slit-widths must be used in order to enable sufficient light to fall on the photocells. It is not only that the stray light is of more influence, but, with a comparison solution, there are two possibilities for deviations of the Beer's law, namely (1) the high concentration (Beer's law is in principle a boundary law applicable to monochromatic light and an infinitely weak solution) and (2) when the width of the slit is great, the spectral bandwidth becomes so great, that the light can no longer be considered monochromatic. A narrower focus is then desirable in order to reduce the width of the slit. It may be said that precision colorimetry is an interesting development, but that all methods must meet the requirements of practical use. Differences in cells can also play an important role and certain precautions must be taken. Furthermore, it is apparent that reasonable reproducibility does not demand great accuracy. Before concluding, mention will be made of some results of an investigation by Caster (4) into the various factors which are responsible for the variations in the Beckman spectrophotometer. This examination is important, because it draws attention to factors, which are generally ignored. Failure to set the "dark current" exactly at zero, led to an error of approximately 0.4% per scale division. Therefore in order to obtain reliable measurements the "dark current" must not only be correctly compensated, but must also remain constant. To obtain this stable "dark current" the accumulators must be in good condition and the various contacts must be clean. In view of

the fact that both photocells are usable at 6,100 Å, the differences in these were also investigated. The red sensitive photocell gave at this wavelength a 3.7% greater extinction for a 3% $CuSO_4$ solution, than the cell sensitive to ultraviolet. In the long run, the red cell was also less stable than the ultraviolet cell. After a time lapse of eight months, the differences were respectively 2% and 0.6%. Thus this factor can have a serious effect on the accuracy of absolute measurements.

CONCLUSIONS

(1) It is very desirable that the spectrophotometric instrument should be checked at regular intervals. This can be done by means of a standard solution of known extinction, but it is better to work with standard glasses of accurately known transmission (12)(25). The influence of the cells is then eliminated. It is a disadvantage, that these standard glasses are expensive and difficult to obtain. As a secondary standard use could be made of solutions of, for instance K_2CrO_7. Nevertheless, standard glasses for different wavelengths should be available since cell—corrections must be determined for different slit widths and wavelengths. Preferably transmissions between 20 and 60% should be used.

(2) Deviations from Beer's law can also be caused as a result of non—linearity of the instrument. It is clear that the fairly large unavoidable errors, may suggest a deviation in Beer's law. This in reality does not exist. If the ultraviolet cell gives in practice a constant molar extinction coefficient for 1%, 3% and 9% $CuSO_4$, then the red cell gives a difference of 2% (4). If extinctions smaller than 0.1 (therefore in the inaccurate region) must be measured control of the law is impossible in practice (26). In that case cells of different optical path lengths should be available.

(3) Too little attention is paid to the error which arises because the solutions are clouded. Particularly if "old" solutions are involved these should be centrifuged. Also turbidity, which is not perceptible to the eye, can be the cause of error amounting to a few percent.

(4) By careful operation the error with comparative measurements can be brought down to approximately 1%. This applies to methods of analysis in which comparison is always made with a known standard, for instance a known solution.

(5) In the determination of absolute molecular extinction coefficients, errors of at least 5—10% should be expected. A similar error can occur in an analytical determination, in which calculation is made from the measured extinction ($\epsilon C l$) the concentration C and a value for ϵ taken from the literature.

REPRODUCTION OF EXTINCTION CURVES

In reproducing extinction (absorption) curves, it is desirable to fulfil the following conditions:

(1) Integrate log ϵ against λ (in Å's) where λ increases towards the right. Log ϵ gives a better representation because the variation in ϵ is large in most cases. Often the wavelength is expressed in m μ since the wavelength is accurate approximately to one m μ. However, apart from the Å, it is perhaps better to use no other unit for atomic dimensions, but to adhere to general spectroscope practice even though this results in the last digit being generally a zero. The actual points of measurement should be indicated on the curve.

(2) The following should also be mentioned:

A. the concentration relative to Beer's law,

B. the solvent. Cyclohexane and methyl cyclohexane are good solvents for aromatic compounds. For more polar materials, 95% alcohol may be used (not the absolute alcohol, as this is often contaminated with traces of benzene). For materials, which dissolve with difficulty; 1.4 dioxane can be recommended, provided this is well purified. A list of solvents that can be used and their degree of purity is given in reference 27.

C. Temperature. The extinction curve varies with the temperature, at the rate of 0.1% per degree centigrade. In this case the reduction in concentration as a result of expansion of the fluid must be taken into account.

D. The width of the slit or, if possible, the spectral bandwidths.

E. The length of cell and the type of the instrument used.

LITERATURE

This article, without concentrating too much on details of operation gives a general review helpful to the investigator working in the field of ultraviolet spectrographic analysis. Among the literature previously mentioned, the articles by Hogness et. al. (13), Caster (4), Gibson and Balcom (12) and Bastian et. al. (18) are specially important. Furthermore "Colorimetry and Spectrophotometry" by G. Kortūm can be recommended, although the modern American instruments are dealt with too summarily (19). In the first 130 pages of the second part of Physical Methods in Organic Chemistry, W. West deals with spectrophotometry in a way which is entirely in conformity with the general high level of this standard work (20). In the January and February numbers of Analytical Chemistry (also obtainable separately) from 1949 onwards, there are annual reviews of absorption and ultraviolet spectrophotometry. In these reviews very many publications are represented. This applies also to the book by Sandell (23).

We are grateful for the co-operation of the Laboratory for Universal and Inorganic Chemistry and for Analytical Chemistry, in particular to Professor Dr. J.A.A. Ketelaar and Professor Dr. W. van Tongeren for their assistance in compiling this review.

DISCUSSION

J.A.A. KETELAAR

The cleanliness and preparation of the cells are important factors. The relative accuracy of measurements by one person with one instrument of one material is certainly higher than that which may be evident from the "test". As regards the problem of the choice of a material for comparison, there are indeed only very few materials, for which the extinction is known with satisfactory accuracy.

H.W. DEINUM

Experience teaches us, that the ultraviolet Beckman spectrophotometer can be used reasonably well to 1,600 mμ, so that, the higher frequencies of carburetted hydrogen can be measured correctly. The "automatic" Beckman discussed in the lecture is a very expensive instrument (price approximately 40,000 guilder).

P.B. ROTTIER

Should not a "test solution" such as $K_2Cr_2O_7$ be used regularly, in order to keep the user of an ultraviolet spectrometer up to date, regarding the condition of his instrument?

ANSWER

As stressed in the present paper the regular checking of the ultraviolet instrument is a constant requirement. However, the problem is, how can this best be done. In the author's view the best method is to use a set of standard glasses of known transmission.

W.D. DEYS

Has not the $K_2Cr_2O_7$ been wrongly chosen as a standard for comparing the accuracy of various instrument (influence pH, easily reducible; 0.01 N H_2SO_4 is also not buffered)?

ANSWER

$K_2Cr_2O_7$ is chosen principally because it possesses favourable physical properties, as mentioned in the article. Indeed, one may expect that it will show some chemical instability, so that care must be taken that any contact with easily oxydisable materials is avoided. At the Laboratory for Analytical Chemistry, research is proceeding regarding the effect on the extinction of $K_2Cr_2O_7$ contaminated with H_2SO_4 (20).

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solution of $K_2Cr_2O_7$ prepared in this way, retains a constant extinction for a period
of one week. The transmission, after three weeks, over the whole region has, on an
average, increased by 2–3%.
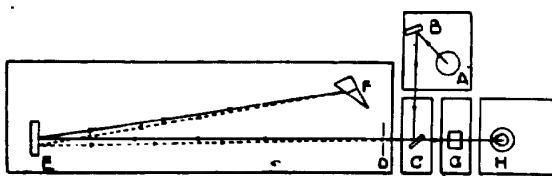
Fig.1: The optical system of the Beckman
spectrophotometer
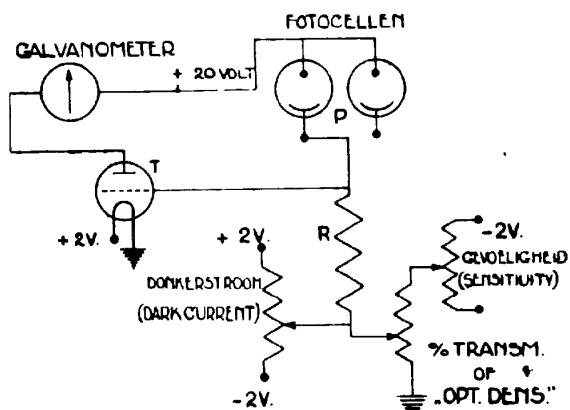

Fig.2: Simplified circuit of the Beckman
spectrophotometer
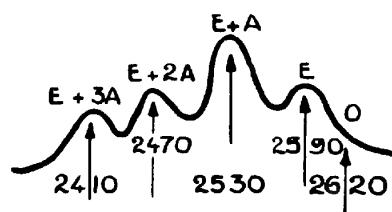
ABSORPTION CURVE
(Schematic)

A - VIBRATION   E - VIBRATION
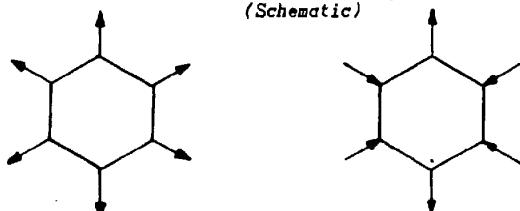
Fig.3: Band system for benzene at 2600 Å
Ground state 992 cm^{-1} Ground state 606 cm^{-1}
Excited state 923 cm^{-1} Excited state 520 cm^{-1}

II.   CRITICAL INVESTIGATION OF SIMPLE
SPECTROPHOTOMETERS

R. Schmidt

1.   INTRODUCTION

This paper describes certain investigations which have been undertaken at the Analytical Institute, T.N.O., for the purpose of obtaining some knowledge of the reliability of simple spectrophotometers, which are universally in use in analytical practice.   The spectrophotometers, which are examined, are not very interesting as such, unless they are examined in order to investigate certain principles of measurement.   The emphasis is upon the critical evaluation of the observations , a problem which generally is more involved than the instrument itself.   Similar investigations are at the moment very much in vogue, witness the many "collaborative tests" which are undertaken.   However important such tests may become it is my impression that many of the individuals participating have no thorough knowledge of the instrument, and worse still do not maintain their instruments in good condition.   This conclusion is in agreement with the recent decision of the TAPPI (Technical Association of the Pulp and Paper Industry) to be very wary of such large scale researches, since they produced more confusion than clarification.   In my opinion, it would be preferable for investigators to study more seriously the properties and capabilities of the various instruments and the correct methods to be used in research.

The ultimate aim of this investigation is to answer questions regarding the best method of measurement particularly with regard to accuracy in measuring the optimum extinction (absorption).

2.   NATURE OF POSSIBLE ERRORS

It is clear that an insight into the behaviour of an instrument can only be obtained by repeated measurements, under clearly prescribed circumstances.   The instrument must, as it were, itself indicate what it can and what it cannot perform.   The setting up of the test must preferably adhere, as near as possible, to general practice.   In addition it is necessary to take into account the character of the sources of error, whether they are systematic or fluctuate.   It seems that there is also a hybrid form of error, namely systematic errors, which build up and finally become subject to fluctuation.   Although it may sound absurd, we have been able with an objective spectrophotometer, to ascribe such a case to the subjective influence of the investigator.   Eventually, these fluctuating systematic errors become ordinary random errors (fluctuations).   It really means that the instrument, with which they are observed, are not sufficiently tested and calibrated.

When determining the magnitude of the fluctuations, in the results obtained with a specific instrument, it is essential that the required measurements are undertaken at random, i.e. in such a way that the sequence of the observations may in no way be connected with the systematic continuity of the observations.   As an example consider the setting up of a standard line for a spectrophotometric analysis.   Assuming that we wish to set up this line on the basis of measurements, undertaken in triplicate, of five standard solutions, it is incorrect to undertake the observations in the direction of increasing or decreasing concentrations of the standard solutions or to undertake the repetitions in succession with one concentration.   The correct method is to arrange the order of sequence by chance from the total number of observations to be performed.   The design standard V.1047, reproduction of series of observations, gives the order of sequence required (Chapter 4).

When determining fluctuating systematic errors, it is essential to use a different technique and to repeat the series of observations, singly and at random several times.

3. TYPES OF SPECTROPHOTOMETER

Besides the character of the sources of errors, the method of measurement is of importance. The method to be used depends on each individual case and no general rules can be laid down.

Spectrophotometers are divided into three groups:-

(a) deflection type (Coleman Jr.)

(b) compensation type (Nedoptifa)

(c) substitution type (Spekker absorptiometer).

The principles of measurement can be represented schematically as follows:
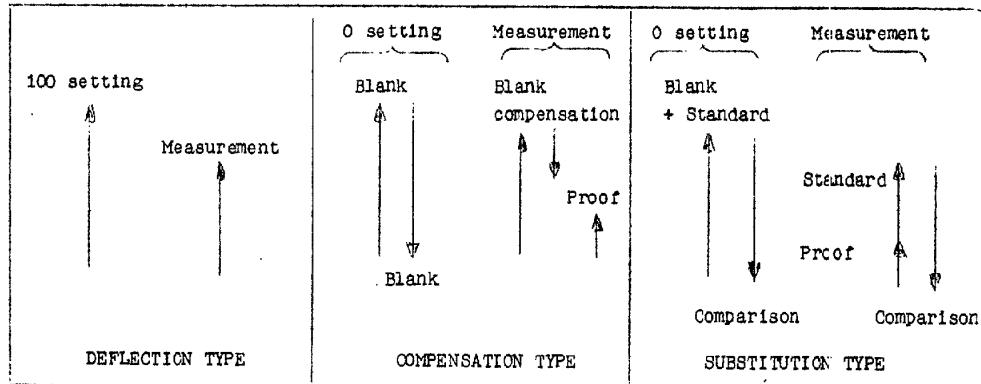
The possibility of using a comparison solution with a final extinction (absorbance), instead of a blank should be considered.   This amounts to an "expansion" of the galvanometer scale as regards the first type instrument.   In this way, the optimum extinction becomes variable.

4. THE ROLE OF STATISTICS

In investigations of this kind, it is essential to take into account the fact that fluctuations are superimposed on the systematic and the fluctuating systematic errors.   It is almost impossible to unravel this knot without making use of modern statistical methods (standard deviation, variance, spread).

It is essential to take into account   the form of the frequency distribution of the errors.   These are certainly not, in all cases, of the normal Gaussian type.   For instance, a rectangular distribution may be encountered, i.e. distribution by which inside a certain interval each error has the same a priori probability, and outside this interval a probability 0, e.g. errors in reading divisions of the scale.

For this reason, modern methods which waive any supposition of the form of the frequency distribution, the so-called rank correlation methods (2), are very important for this type of investigation.   A few applications to spectrophotometric problems have been published (3).

5. DISCUSSION OF ERRORS

A discussion of errors, based on the Gaussian propagation law for fluctuations, is essential for the interpretation of the fluctuations found experimentally.

A starting point must be the expression for the extinction, as a function of the magnitudes actually measured (for instance galvanometer readings with spectrophotometers of the deflection type).

Assuming that the extinction E is a function of the experimental magnitudes x, y and z

$$E = f(x,y,z),$$

the variance of E (square of the standard deviation) is then given by

$$S^2(E) = \left[\frac{\delta f}{\delta x}\right]^2 S^2(x) + \left[\frac{\delta f}{\delta y}\right]^2 S^2(y) + \left[\frac{\delta f}{\delta z}\right]^2 S^2(z)$$

- 16 -

To this must be added the contributions of factors, which fall outside the functional relation such as errors in scale reading.

These observations can lead to an evaluation of the relative significance of the various sources of fluctuations and thereby point the way to improvements in the construction and use of the instrument. As a general rule, the important point is that a partial variance, which is four times or more as great as the other put together, is the dominating factor.

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III.   THE BEHAVIOUR OF THE SPEKKER ABSORPTIOMETER

R. Schmidt

The Spekker absorptiometer has been critically evaluated in its two models H.560 and H.760.

It has been conclusively shown that neither of the two models exhibits a linear characteristic of the drum diaphragm when equiped with a tungsten lamp.   This causes systematic differences between the results obtained on the same solutions with the two principles of measurements, proposed by the manufacturer and by Vaughan respectively. Since this is not the case with a H.560 instrument equiped with a mercury lamp (except when the diaphragm is wide open) the cam which actuates the diaphragm cannot be held responsible.   The difficulties are linked up with the tungsten lamp, which is also responsible for significant calibration curve drifts.   Within equivalent calibration curves the standard deviation of one measurement is about 2.5×10^{-3} irrespective of absorbance (up to 0.85), wavelength or principle of measurement, provided the galvanometer is sufficiently sensitive.

1.   DESIGN OF THE INSTRUMENT AND PRINCIPLES OF MEASUREMENT

The Spekker absorptiometer is an instrument of the substitution type for which a schematic diagram is given in Figure 1.

The substitution is obtained by means of the combination cell and the measuring diaphragm, which can be adjusted.   The photocurrent, which at the measuring side of the instrument, is generated by the photocell, is compensated by the photocurrent, which is generated by a second photoelement with variable diaphragm.   The method of measurement must be independent of variations in the light current.   This requirement is met provided the ratio between the transmissions of both halves of the instrument and that of the response of both photocells are independent of the light current and the spectral distribution of energy (1)[*].   Two methods of measurement have been given by the firm Hilger (2) and by Vaughan (3) respectively.

Hilger: Zero setting with measuring diaphragm fully open and test solution in the cell: blank measurement with measuring diaphragm adjusted until the galvanometer again reads zero.

Vaughan: Zero setting with blank cell and measuring diaphragm opened by 1/10: measurement with test solution in the cell and the measuring diaphragm adjusted until the galvanometer again reads zero.

The manufacturers maintain that using their method a greater accuracy is obtained due to the fact that the reading is more accurate.   As will appear from what follows, it is not possible to uphold this claim.   Relatively few objective data are known regarding the capabilities of the H.760 instrument.   Isbell (4) has compared the improvements in comparison with the older types, without furnishing quantitative data.

Pollak and Nicholas (5) have reconstructed a Spekker absorption meter using a mercury lamp for the purpose of obtaining greater accuracy.   This paper, however, gives little positive information.

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[*]   For references, see page 20.

- 18 -

2. INSTRUMENTS USED AND EXPERIMENTAL TECHNIQUE

Observations were made with two types:

(a)   an older type (H.560) equiped with a tungsten lamp, as well as a mercury lamp, and

(b)   the latest type (H.760) with a tungsten lamp.

The older instrument was equiped by us with a Kipp A75 galvanometer with adjustable sensitivity, so that under any condition it can be operated with the same sensitivity for zero indication.   In addition, a 500 watt resistance of 5 ohms was connected, in series, with the tungsten lamp, in order to prolong the life of the lamp.   All measurements were made with suitable concentrations of a grey solution prepared from

16.7 gr.	potassium chromium (III)-alum
33.3 gr.	copper sulphate
39.5 gr.	ammonium cobalt (II)-sulphate (6 H_2O)
0.12 gr.	potassium dichromate

dissolved to 1 litre.

3. EFFECT OF VOLTAGE FLUCTUATIONS ON THE LAMP

From many series of observations in which the tungsten lamp was fed via a variac, by which the voltage was varied between 170 and 230 V it appeared that the voltage had no influence on the measurement.   The standard deviation of the extinctions obtained "between voltages" was, for none of the filters, greater than values obtained normally over a short period with one voltage (approximately 3×10^{-3} extinction).   Even alteration of the voltage between zero adjustment and the measurement had no effect.   The construction of an instrument, which is independent of the fluctuations in the light source has therefore certainly been achieved.

4. CHARACTERISTICS OF THE MEASURING DIAPHRAGM

The heart of the instrument is the measuring diaphragm, which is characterised by the following expression

$$i_m (\alpha) = A \alpha = A \, 10^{-R}$$

in which

$i_m (\alpha)$ =   photocurrent in the galvanometer supplied by the photocell behind the diaphragm for a relative opening α of the diaphragm.

A =   a proportion factor.

R =   the reading on the diaphragm scale.

This characteristic can be simply determined by setting the measuring diaphragm at a value desired, setting the galvanometer with the left-hand diaphragm at zero and finally by covering up the left-hand photocell.   The galvanometer will then indicate the photocurrent, which is generated in the measurement side (i_m).

The characteristics defined in this way for the tungsten lamp and the mercury lamp are different (Figure 2).   The curve for the tungsten lamp is non-linear, whereas that for the mercury lamp, is practically linear, except at openings in the neighbourhood of 1.0.   On repeating the measurement, the characteristic of the tungsten lamp shows a very pronounced systematic variation between 0.4 and 0.8 (with the H.560 model).   On the contrary, the characteristic of the mercury lamp, shows a random variation neglecting the non-linearity at large apertures. All this is reflected in the calibration curves determined for the instrument.   When using the tungsten lamp, the calibration curves are non-linear.   In addition the calibration curves differ because of the curvature of the diaphragm.   (According to measurements made by Hilger and Vaughan).

When using the H.760 model, tested by us it has been found that the calibration curves differ significantly for the two methods of measurement. From this fact it may be concluded by analogy that here also the characteristic of the tungsten lamp is not linear. While, however, the curve for the model H.560 is concave in relation to the aperture axes, that for the H.760 model must be convex in relation to this axis (Figure 3) The difference in behaviour between the tungsten and mercury lamp is a proof, that the cam, which actuates the diaphragm is correctly constructed.

5. FLUCTUATING SYSTEMATIC DIFFERENCES

By means of a technique described elsewhere, and a parameter free statistical investigation of the results obtained with the H.760 model, it can be demonstrated that this model shows fluctuating systematic differences (calibration curve drift) if the tungsten lamp is used. That means that the calibration curves are not all of equal value, and cannot be used indiscriminately. In a somewhat different way, the same thing is demonstrated for the H.560 model.

On the contrary, with the H.560 model using a mercury lamp, there are no signs of any significant drift of the calibration curves.

It is quite justifiable to relate this association between the drift of the calibration curve and the use of the tungsten lamp with the systematic deviations in the characteristic of the measuring diaphragm. This claim is supported by the fact, that it has not been found possible, using random observations, to obtain consistent estimates of the standard deviation of the extinction measurement and especially in the region where systematic deviations of the diaphragm characteristic were noticeable.

No absolutely valid explanation of these systematic deviations has yet been found. A possible cause is the existence of extraneous light in the measuring diaphragm, due to reflections from the filter, lens and the lamp holder.

6. RANDOM DEVIATIONS (FLUCTUATIONS)

From the data relating to four series of nine calibration curves determined with varying concentrations of the grey solution for different methods of measurement and different filters, the standard deviation at one observation was calculated as a function of the extinction for each method of measurement using two filters (Ilford 602 and 607). It appeared from this calculation, that the standard deviation is independent of the filter and of the method of measurement employed and depends only on the extinction. The standard deviation increases from 3×10^{-3} at extinction 0 to 8×10^{-3} at extinction 0.8.

In this calculation, the fact that not all the calibration curves of one series are interchangeable was neglected. If we calculate the standard deviation for equivalent calibration curves, then it appears that the dependence on the extinction disappears, so that up to the highest extinction measured, viz. 0.85, a uniform standard deviation of 2.5×10^{-3} is obtained provided the galvanometer is sufficiently sensitive[*], (Figure 4). This fact is very noticeable because, with all conceivable sources of fluctuations (reading zero adjustment, manipulation of the diaphragm, galvanometer reading) we expect an exponential relationship between the reading of the diaphragm scale and the standard deviation. On the other hand, the standard deviation thus found, is approximately equal to that mentioned earlier in connection with the effect of varying the voltage of the tungsten lamp (Section 3).

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[*] This is the case when the displacement of the measuring diaphragm from the position corresponding to zero current through the galvanometer required to give a higher reading of 0.1 to 0.3 extinction units on the extinction scale will at the same time produce a galvanometer deflection of half the full scale length of a Cambridge or de Kipp galvanometer.

- 20 -

REFERENCES

(1)   See also R. H. MÜLLER in M. G. MELLON Analytical Absorption Spectroscopy, New York, London 1950, p. 173-174.

(2)   See Ref. 4.

(3)   See, amongst others, F.W. HAYWOOD, A. A. R. WOOD. Metallurgical analysis by means of the Spekker Photoelectric Absorptiometer.   London (Hilger).

(4)   R. A. C. ISBELL. Analyst. 74 (1949) 618.

(5)   F. F. POLLAK, J.W. NICHOLAS. Metallurgia 44 (1951) 319.

K_V $D_V F_V$ C_V L C_M $F_M D_M$ K_M
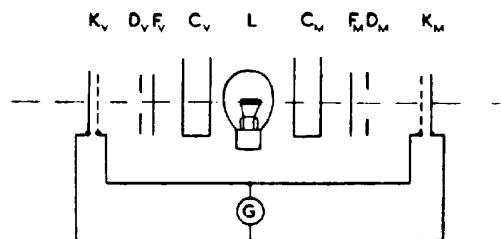
Fig.1: Schematic diagram of the Spekker Absorptiometer
L = lamp; C = cell; F = filter; D = diaphragm;
K = photocell.   The indices v and m correspond to
the comparison and measuring sides respectively.

H560 Tungsten

H560: Mercury
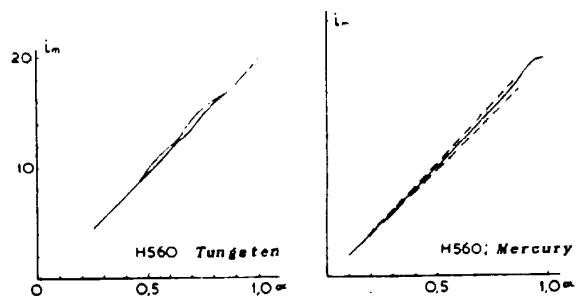
Fig.2: Reproducibility of the measuring diaphragm
characteristic of the Spekker.

VAUGHAN

HILGER

HILGER

VAUGHAN

HILGER

HILGER

VAUGHAN

H560 Tungsten

H760 Tungsten

H560 Mercury

CONC
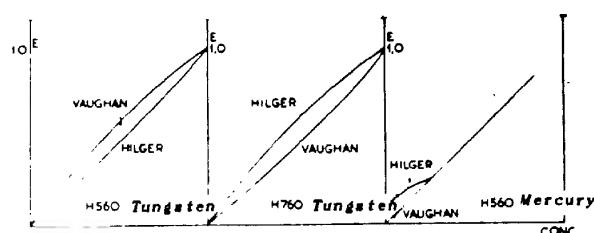
Fig.3: Calibration curves according to the methods
of Hilger and Vaughan for different instruments.

$S[E] \cdot 10^8$

All calibration curves

Equivalent calibration curves

0,5

1,0 E
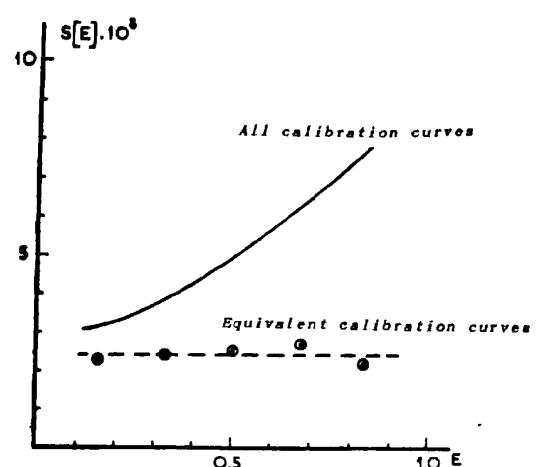
Fig.4: Reproducibility over long period of the extinction
measurements as a function of the extinction expressed as
a standard deviation of the extinction within equivalent
calibration curves.

IV. PRECISION EXTINCTION MEASUREMENTS WITH SIMPLE SPECTROPHOTOMETERS OF THE DEFLECTION TYPE

J. H. L. Zwiers

Chemisch Weekblad 49 (1953) 496-499

(From Dutch)

This paper discusses the derivation and the significance of a formula for the variance of extinctions measured with spectrophotometers of the deflection (Coleman Jr. Model 6A, Engel colorimeter and the like). It is shown inter alia, that the minimum of the variation fraction for the extinction is not generally situated at the value 0.434, but at a value which is determined by the ratio of two of the instrument parameters. This ratio can be simply determined by experiment. The precision of extinction measurements made with a Coleman Jr. Model 6A spectrophotometer is discussed on the basis of the foregoing remarks.

1. A FORMULA FOR THE VARIANCE OF THE EXTINCTION

In order to measure the extinction of an unknown solution with a spectrophotometer of the deflection type, two steps are necessary.

(a) The determination of the galvanometer deflection $u_{ref.}$ for a comparison solution - in practice this means adjusting the spectrophotometer sensitivity so that $u_{ref.}$ amounts to a full scale deflection L -

(b) The determination of the galvanometer deflection u_χ for the unknown solution itself.

The extinction is then equal to

$$E = \log u_{ref.}/u_\chi \qquad \dots\dots (1)$$

Since the factor u is proportional to the photocurrent i of the spectrophotometer detector we get

$$E = \log i_{ref.}/i_\chi \qquad \dots\dots (2)$$

From (1) by differentiation squaring of the differentials and summation of the squares of dE over an infinitely large number of observations an expression can be derived for the variance of the extinction $var_1(E)$ with respect to the reading errors,

$$var_1(E) = 0.434^2 \lambda^2/12 \, L^2 \, (\alpha^2 + 10^{2E}) \qquad \dots\dots (3)$$

in which λ = the smallest perceptible distance on the galvanometer scale and bears the following relationship to var (u)*

$$var\ (u) = \lambda^2/12 \ .$$

α = var (u_{ref})/var (u_χ); α is a constant of $\leqslant 1$ depending on the adjustment u_{ref}, the accuracy cannot exceed that of the reading of u_χ.

L = full length of the galvanometer scale $= u_{ref}$

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*   For references, see page 25.

- 22 -

From (2) and the expression

$$i = A \cdot I \qquad \dots\dots (4)$$

in which A = the integral sensitivity of the detector

I = the intensitivity of the light falling on the detector,

an expression can be derived for the variance of the extinction $var_2 (E)$ in relation to the fluctuations of the sensitivity of the detector and the fluctuations of the intensity I_0 of the source of light, namely:-

$$var_2 (E) = 0.434^2 \{2\ \Phi^2 (A) + 2\ \Phi (I_0)\} . \qquad \dots\dots (5)$$

where $\Phi(A) = \sqrt{\overline{A}}/A$, the variation fraction of A,

$\Phi(I_0) = \sqrt{\overline{I_0}}/I_0$, the variation fraction of I_0.

The total variance $var (E_x)$ is equal to the sum of $var_1 (E_x)$ and $var_2 (E_x)$. This sum may be expressed in the form

$$var (E) = P + Q \cdot 10^{2E} . \qquad \dots\dots (6)$$

It should be noted that this formula applies for the method of measurement using either a blank or coloured comparison solution. In the first case E represents a true extinction, in the second case, a relative extinction.

If "true" extinctions are indicated with an asterisk, then

$$E^* = E + E^*_{ref}.$$

2. INTERPRETATION OF THE FORMULA

Equation (6) shows the following:

(a) the magnitude of $var (E)$ for a given value of E is defined by the magnitude of P and or Q,

(b) $var (E)$ does not change with the extinction if Q is small in comparison with P, that is if $\Phi(A)$ and $\Phi(I_0)$ deminate over λ/L,

(c) $var (E)$ is an exponential function of E if Q is equal to or greater than P, that is if λ/L dominates,

(d) the variation fraction of the extinction $\Phi(E)$ has a minimum.

The value of extinction near which this minimum is situated, can be found by equating the derivative in E

$$\Phi(E) = 1/E \sqrt{P + Q \cdot 10^{2E}}$$

to zero giving

$$E \ln 10 - 1 - P/Q \cdot 10^{2E}$$

The value of E which satisfies this expression is determined graphically as the point of intersection of the curve

$$\gamma_1 = E \ln 10 - 1$$

with the curve

$$\gamma_2 = P/Q \cdot 10^{-2E}$$

See Figure 1.

The position of the minimum of $\Phi(E)$ is therefore determined by the magnitude of the ratio P/Q and is situated near extinction 0.434 where $P/Q = 0$, provided that the observation fluctuations are attributed exclusively to errors in the reading the values of u_x. With an increase of P/Q (therefore with an increase of $\Phi(A)$, $\Phi(I_0)$ and α) and/or a decrease of λ/L (i.e. with smaller reading errors) the minimum shifts to greater extinction values.

3. DETERMINATION OF THE INSTRUMENT PARAMETERS

With a specific spectrophotometer, the values of the parameters P and Q can be estimated very simply experimentally.

In this way information is obtained regarding the type and importance of the sources of error associated with this instrument and in addition the conditions which must be fulfilled in order to obtain the utmost precision of measurement become known. As an example the results of an investigation of a Coleman Jr. spectrophotometer model 6A are given.

A great number of extinction measurements were made at various wavelengths in random sequence. Each extinction value was measured in duplicate. The variance var (E) was calculated for each of the ten observations. Var (E) was plotted against 10^{2E}. See Figure 2.

As an approximation of the mean values of P and Q the intercept and the regression coefficient respectively of the line of regression drawn in Figure 2, are determined by means of the method of least squares.

In this way we get a small negative value for P of $- 0.24 \times 10^{-6}$ and a value of 0.28×10^{-6} for Q. Thus in the case of the Coleman Jr. model investigated the effects of the inconstancy of the sensitivity of the detector of the inconstancy of the intensity of the light source, and of the errors in the adjustment of u_{ref} to L included in P can be neglected with respect to the effect of inaccurate reading, included in Q. In addition the minimum of $\Phi(E)$ in this case is still situated near 0.434.

In Figure 3, $\Phi(E)$ is plotted against E for $P = 0$ and $Q = 0.28 \times 10^{-6}$.

The curve shows inter alia that:-

$\Phi(E)$ mean $\leqslant 0.5\%$, if $0.16 \leqslant E \leqslant 0.95$

$\Phi(E)$ mean $\leqslant$ 1%, if $0.06 \leqslant E \leqslant 1.43$.

Therefore accurate measurements can be obtained over a fairly wide extinction region. It should be emphasised that in this case E represents, in general, a relative extinction. If the "true" extinction of the comparison solution amounted to 1.00, solutions with a "true" extinction of 1.95 could be measured with a $\Phi(E) \leqslant 0.5\%$.

4. SUBJECTIVE FACTORS

The points plotted in Figure 2 show a very substantial spread. An investigation to discover whether any unanimity existed in the variance of E found with approximately equal values of E by means of the so called Bartlett test, indicated that generally speaking, there was no unanimity (the values did not lie in a single frequency region). The explanation for this unexpected phenomena seems to be in the fact that the magnitude λ, the just perceptible distance, on the galvanometer scale is not a constant but a stochiastic variable.

In order to verify this assumption the value of λ for each value of var (E) was computed from

$$\text{var } (E) = Q \cdot 10^{2E} = 0.434^2 \lambda^2/12 \, L^2 \cdot 10^{2E}$$

(With the assumption that $P = 0$).

By grouping the λ values for various investigators (P, H, Z and B) into histograms, it appeared that λ may be indeed considered as a stochiastic variable with an approximate normal frequency distribution, and a rather large standard deviation $S(\lambda)$.

It is probable that the mean magnitude of λ, that is, $\bar{\lambda}$ and the standard deviations of λ were influenced fairly strongly by the capability of the observer. See Figure 4.

In Table I the values of $\bar{\lambda}$ and $S(\lambda)$, are given for the results of one observer B and for the combined results of the observers P, H and $\dot{Z}$.

TABLE I

OBSERVER	$\bar{\lambda}$	$S(\lambda)$	NUMBER OF DEGREES OF FREEDOM
B	0.40 mm	0.17 mm	27
$P + H + Z$	0.69 mm	0.28 mm	27

$S(\lambda)$ is not only great in comparison with $\bar{\lambda}$ but also $\bar{\lambda}$ itself is fairly large. This is probably due to the fact that the scale divisions of the Coleman Jr. are indicated with fairly thick lines, while the pointer itself is fairly wide. The precision of the instrument could also be increased by extending the length of the galvanometer scale. For example, in order to obtain var $(E) \leq 10^{-6}$ for $E \leq 1.00$ the scale length should be made equal to:

$$L = 125\,\bar{\lambda}\ \text{mm.}$$

DISCUSSION

H. COX

What is the position with regard to observation fluctuations in connection with the connection with the galvanometer readings of the Coleman Jr.?

ANSWER

The fluctuations in the galvanometer deflection caused by Brownian movement and Johnson murmer are, in the case in question, negligibly small. The galvanometer of the Coleman Jr. is robust and not extremely sensitive. Apart from the fact that one could consider these phenomena as already included in the fluctuations of A, one should visualise A as the integral sensitivity of the whole detection system (photoelement + galvanometer). For that matter, from equation (2), similar fluctuations may easily be expressed as a factor $\text{var}_{3}\ (E)$.

J. de WAAL

The values of P and Q in the formula var $(E) = P + Q\ 10^{2E}$ are of the same order of magnitude although P is negative. Is it correct in this case for P to be taken as zero?

ANSWER

This is indeed the case. Perhaps I can best make this clear as follows:-

If the line of regression drawn in Figure 2, is rotated about its centre so that it passes through the point of origin, the sum of the squares of the distances of the points from the line and also the magnitude of Q remain almost unchanged whereas P has become equal to zero.

A. van KREVELD

The speaker attributes the frequency distribution of λ for each observer to a difference in behaviour of the observers from day to day. Is not rather the frequency distribution attributable to the fact that each λ corresponds to the spread of only ten observations of E, and is therefore itself of low accuracy?

ANSWER

A measure for the spread of λ is found in $S(\lambda)$ which is large in comparison with the values of λ itself. This is due to the strong inhomogenity of the values of var (E). It is possible that with an increase in the number of observations of E, the values of both λ and $S(\lambda)$ will decrease, it is not to be expected that at the same time the ratio of these magnitudes will also decrease (i.e. that the inhomogenity of the values of var (E) will disappear).

J. van DRANEN

To what extent does the preference of the observer for certain numbers play a role in your observations when reading in values of tenths of a scale unit? It is a psychological fact that numbers such as 7, 2, 8 and 1 enjoy a definite preference.

ANSWER

It would seem difficult to express this preference explicitly in formula (6). Nevertheless, the effect is implicit in the factor Q.

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(1)   L. A. COOPER.   Errors in scale estimation, Research 1, 624 (1948).

E. L.
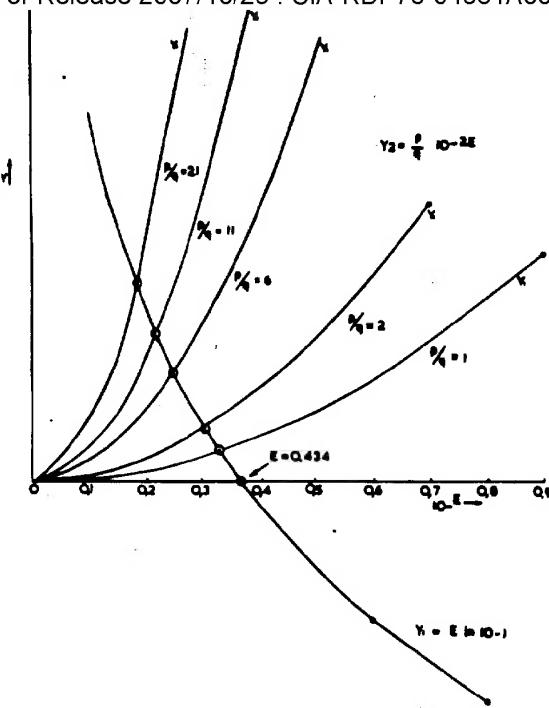
Fig.1: Graphic determination of the minimum of $\Phi(\epsilon)$.
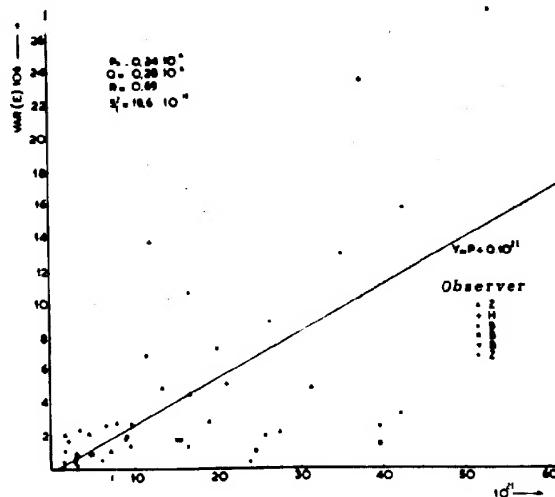
Fig.2: Determination of the instrument parameters
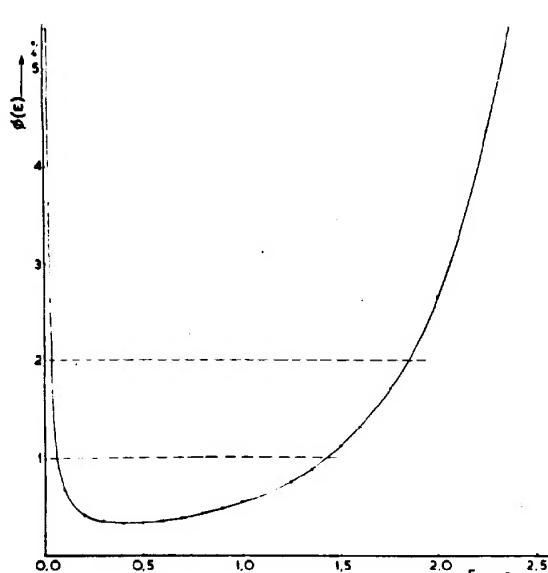
Fig.3: The average variance fraction of ϵ as a function of ϵ for

Fig.4: Histogram for λ for various observers